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(54) Title: SYNTHESIS OF TRIPHENYLPHOSPHONIUM QUINOLS AND QUINONES

(57) Abstract: The methods of preparing quinols and quinones typified by mitoquinol and mitoquinone where a compound typified by idebenone is reacted with Ph₃PHX and Ph₃P, where X is a halogen atom.



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Synthesis of TriphenylPhosphonium Quinols and Quinones

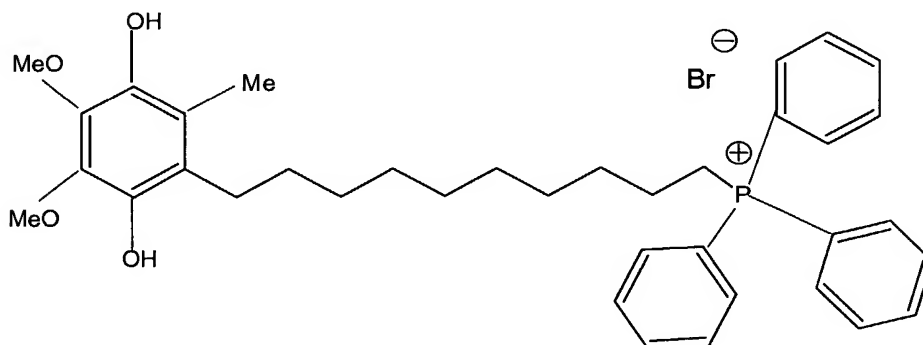
The present invention relates to the synthesis of triphenyl phosphonium quinols and quinones such as mitoquinol and mitoquinone.

In the Journal of Biological Chemistry, Vol.276, No. 7, 16 February 2001, pp 4588 - 4596, Kelso et al. "*Selective Targeting of a Redox-active Ubiquinone to Mitochondria within Cells*" there is disclosed a utility for mitoquinol as a targeted antioxidant for use in the mitochondria of cells, a method of synthesis of mitoquinol and the oxidative changes of mitoquinol to mitoquinone. See also US Patent 6331532.

The full content of that publication is hereby here included by way of reference.

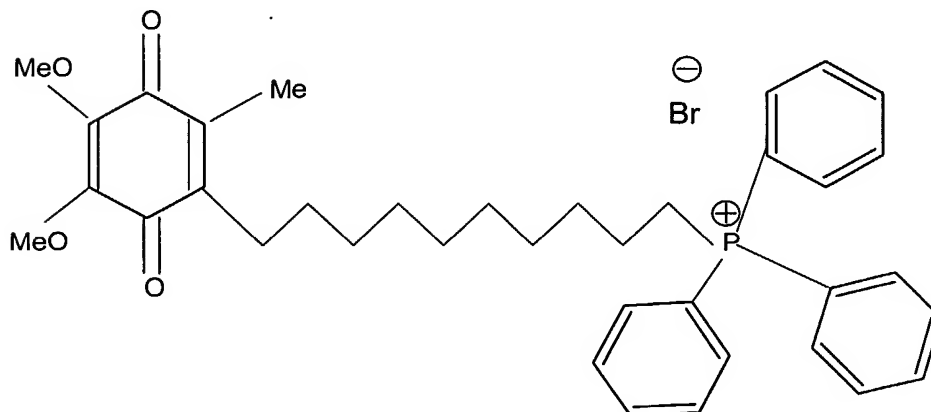
Mitoquinol has the following structure

(Formula IIIA)



Its oxidised form is mitoquinone which has the structure

(Formula IIIB)

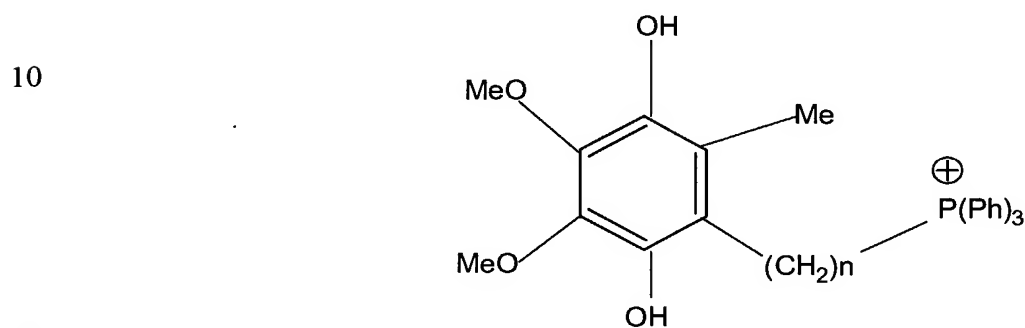


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The present invention relates (in a preferred form) to an alternative synthesis of mitroquinol, mitroquinone, or mixtures of mitroquinol and mitroquinone. It also relates more generally to the synthesis of similar carbon chain linked triphenyl phosphonium and quinol and/or quinone compounds.

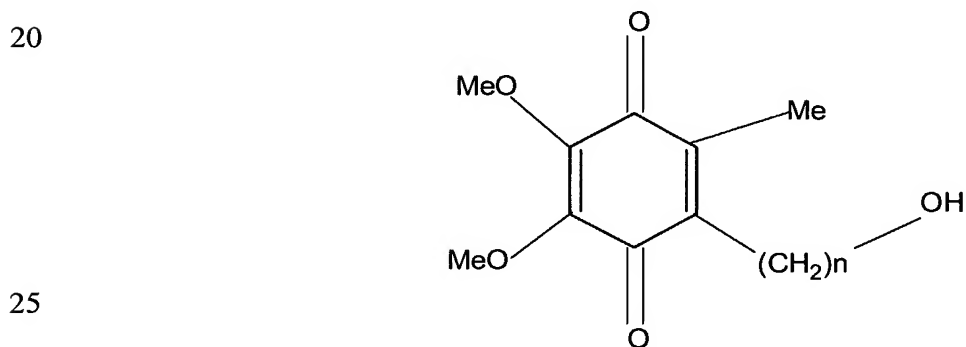
5 In one aspect the present invention consists in a method of synthesis of a compound with a moiety or the moiety of the formula

(Formula II)



(and/or its quinone form) where n is an integer from at least 2 (preferably at least 6) to 40 which comprises or includes the reaction of a compound of the formula

(Formula I)



(and/or its quinol form) in the presence of Ph_3PHX and Ph_3P , where X is a halogen atom.

Preferably X is preferably bromine, iodine or chlorine (most preferably bromine).

30 Whilst n can be from 2 upwards drop for the reaction where n is less than 6 sufficiently to render alternative syntheses more economic.

Preferably n is 6 to 25.

-3-

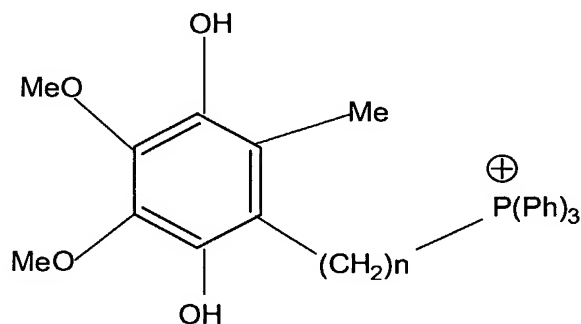
Preferably the reaction is maintained as a temperature below which significant amounts of MePPh_3 are not formed by ether cleavage, eg; the mixture is preferably kept below 80°C .

In still another aspect the present invention consists in a **method of synthesis of a compound with a moiety or the moiety of the formula**

5

(Formula II)

10

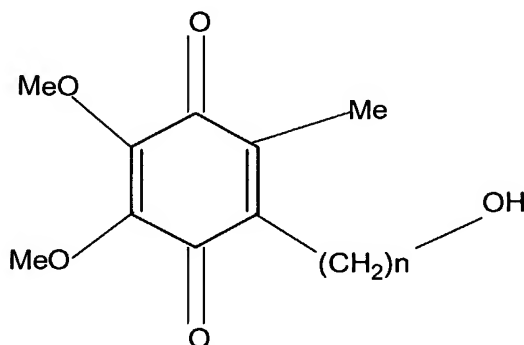


(and/or its quinone form) where n is an integer from 6 to 40 which comprises or includes the preparation or obtaining of a compound of formula

15

(Formula 1)

20



25

(and/or its quinol form) and

its subsequent reaction in the presence of $\text{Ph}_3\text{P}^+\text{HBr}$ and Ph_3P .

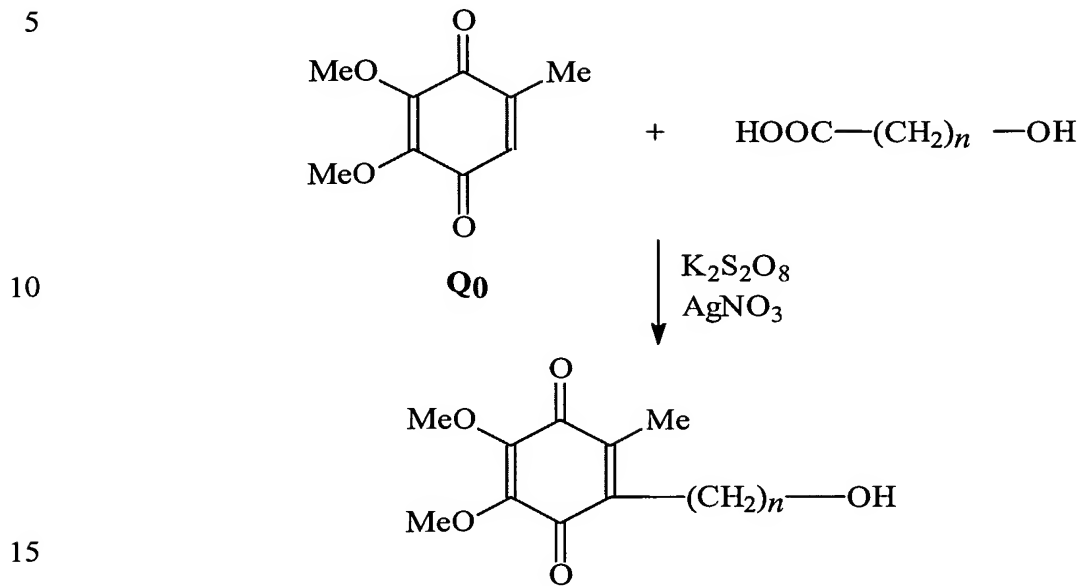
Preferably n is from 6 to 25.

Preferably the reaction is maintained as a temperature below which significant amounts of MePPh_3 are not formed by ether cleavage, eg; the mixture is preferably kept below 80°C .

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By a procedure as follows the starting compounds of Formula 1 where n is from 6 to 40 can be prepared as follows:

(Formula 1)



Yields are 30-40% for n=5,10,15,23 and are based on the readily available starting material (Q_0) and the hydroxyacids - which are well described in the literature.

20 The method is an adaptation of the procedure in JP 08239340 and gives a ready source of the starting materials.

Other approaches to compounds of Formula 1 are by Friedel-Crafts acylation reaction of trimethoxytoluene followed by two reduction steps and quinone formation as described in JP 07223991, EP 0289223. Chemical and Pharmaceutical Bulletin 33(10), 4422-31 1985, JP 59039855, Chemical and Pharmaceutical Bulletin 30(8), 2797-819 1982.

25 Idebenone is a compound of Formula 1 but when $n = 10$.

We have determined that idebenone when reacted with Ph_3PHBr will provide the quinol bromide and Ph_3PO . Yet when Ph_3P is also present in addition the Ph_3PHBr a pathway exists directly through to mitoquinol.

30 The present invention therefore in one aspect is a **method of synthesis of mitoquinol, mitoquinone or mixtures of mitoquinol and mitoquinone** which comprises or includes the reaction of idebenone in the presence of Ph_3PHBr and Ph_3P .

Idebenone is disclosed in §4932 in The Merck Index, 12th Edition.

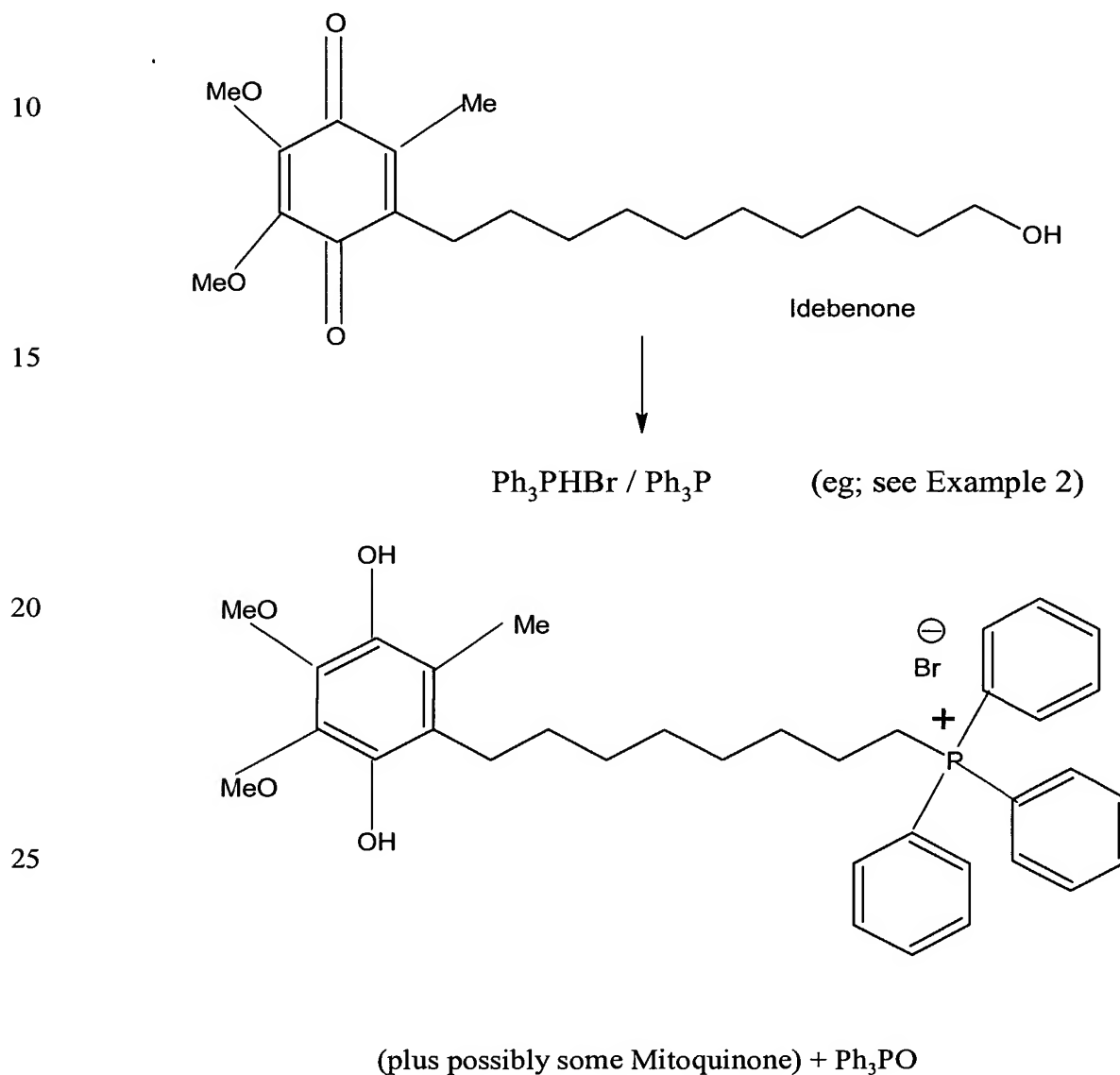
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Preferably the ratio of the idebenone with the Ph_3PBr , the idebenone with the Ph_3P and the ratio of the Ph_3PBr with the Ph_3P is substantially stoichiometric.

Preferably the reaction is maintained as a temperature below which significant amounts of MePPh_3 are not formed by ether cleavage, eg; the mixture is preferably kept below 80°C .

5 In the preferred form of the present invention the reaction through to substantially pure mitoquinol can be described by the following procedure:

(Formula IV)



30

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5

PURIFICATION (eg; see Example 3)
or by vacuum chromatography and filtration)



10

MITOQUINOL (and/or *MITOQUINONE*)
(*Formula IIIA and/or IIIB*)



15

REDUCTION (if needed) (eg; using borohydride)
MITOQUINOL (*Formula IIIA*)

20 Preferably the product that results from the reaction of the idebenone in the presence of the Ph_3PHBr and Ph_3P is mitoquinol (and possibly some of the oxidised species mitoquinone) as well as Ph_3PO .

25 Preferably that reaction product can be purified to substantially purer mitoquinol and/or mixtures of mitoquinol and mitoquinone. For example by washing off with a solvent for Ph_3PO (eg; Et-OAc) and washing with a solvent (eg; H_2O optionally with HBr present) for any phosphonium salts (eg; MePPh_3) or by separation by chromatography.

We have found that it is possible to isolate the material by the procedure hereinafter described by reference to both Example 2 and Example 3 and/or 4.

30 It will be seen that we have found that it is possible with simple EtOAc washing until all of the Ph_3PO has been removed and thereafter a simple water wash (with a presence of HBr) to remove the MePPh_3 (albeit with some loss of the target material) provides purity levels desired, ie; a minimum of 98% mitoquinol (if any mitoquinone present, it is also considered as mitoquinol).

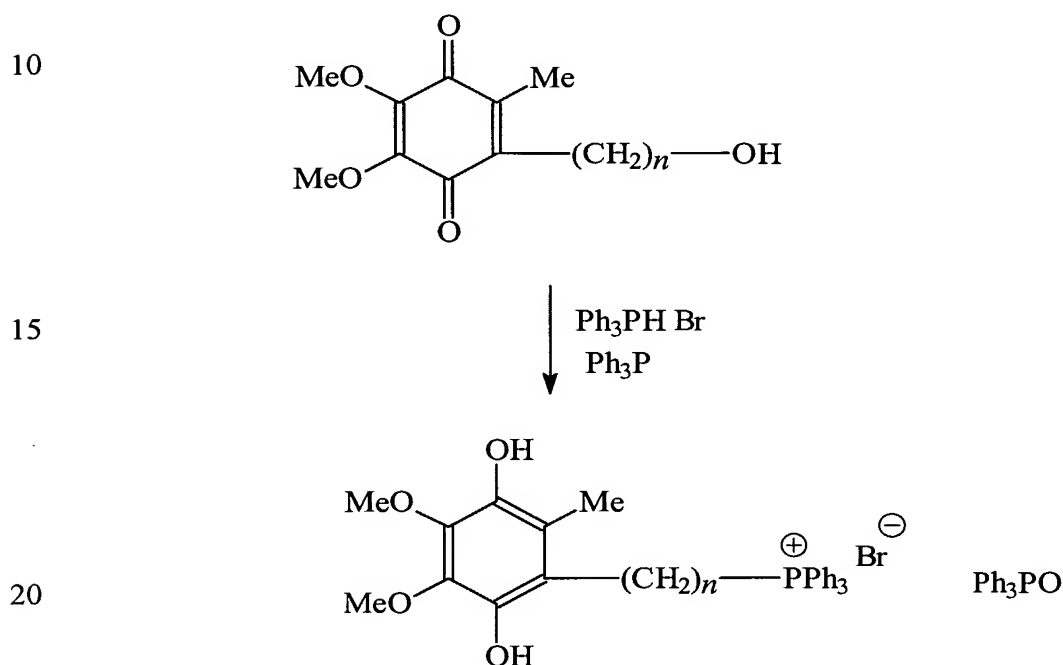
-7-

Alternatively a vacuum chromatography/filtration is possible.

If subsequently needed any mitroquinone present or at least some of the mitroquinone present can be reduced through to the mitroquinol form (eg; using a borohydride).

The present invention also consists in mitroquinol and/or mitroquinone synthesised by any part of a procedure as hereindescribed (including as a precursor or as part of such synthesis of Ph_3PHBr preparation typified by Example 1).

We have determined we can carry out the following reaction for n being 6 and above (eg; to 40):



Yields were low however (eg; for $n = 3$, $n = 5$) when n was below 6.

The present invention will now be further described by reference to the following Examples:

EXAMPLE 1:

Ph_3PHBr PREPARATION

Ph_3P (39.3g, 0.15 mol) was added to 48% aq. HBr (105 mL). The solution was stirred at 70°C for 5 minutes, cooled and extracted with CHCl_3 (3 45 ml).

The combined organic phase was dried over Na_2SO_4 , filtered and the solvent was removed *in vacuo*.

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The residue was washed with warm EtOAc (90 ml); yield: 36.6 g (71 %). (Hercouet, A., Le Corre, M. Synthesis, 157 (1988))

EXAMPLE 2:**5 MITOQUINOL PREPARATION**

Idebenone (0.678 g, 2 mmol, Sequoia Research Products # SRP00400i), Ph_3P (0.524 g, 2 mmol) and Ph_3PHBr (0.686 g, 2 mmol) were placed in a 120x16mm KIMAX tube fitted with a screw cap together with a small TEFLON™ coated spin bar. The tube was flushed with nitrogen, sealed and the bottom 2 cm was placed in a 70°C oil bath on a magnetic
10 stirrer/hotplate with stirring of the mixture. The solids melted quickly to give an easily stirred orange liquid. As the reaction proceeded the mixture became very viscous and turned dark red/brown.

Progress of the reaction was monitored by removing a small sample and recording the ^{31}P NMR in CDCl_3 : $\text{PPh}_3/\text{PPh}_3\text{Br}$ -4.7 ppm, $\text{PPh}_3=\text{O}$ 30.2 ppm, PPh_3Me 23.0 ppm and the
15 product had a peak at 25.6 ppm.

After 16 hours some of the starting materials were still evident but after 22 hours the reaction was complete.

The mixture was then cooled to give a black, glass-like solid which was dissolved in CH_2Cl_2 (4 mL), transferred to a RB flask and the solvent evaporated *in vacuo* to give a dark red
20 oil
(2.446g).

EXAMPLE 3:**PURIFICATION OF MITOQUINOL**

25 The residue from the mitoQuinol preparation of Example 2 (2.446g) was mixed with EtOAc (20 ml) and held at 70°C for 5 minutes then cooled and the solvents decanted. This process was repeated twice more, by which time ^{31}P NMR showed no Ph_3PO remained in the solid residue (1.120g).

The residue (1.120g) was then washed with a solution of H_2O (20 ml) and 48% HBr (3
30 drops) at 60°C for 10 minutes. Any remaining solvent was removed from the residue by evaporation *in vacuo* (0.5mm) to give an orange foam (0.763 g, 57 %). ^1H NMR (299.9 MHz)

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7.6-7.9 (m, 15H, -P⁺Ph₃), 3.88 (s, 6H, 2' -OCH₃), 3.8-3.9 (m, 2H, -CH₂-P⁺Ph₃), 2.5-2.6 (t, 2H, ubiquinol-CH₂-), 2.14 (s, 3H, CH₃). ³¹P NMR (121.4 MHz) 25.7 ppm.

EXAMPLE 4:**5 PURIFICATION OF MITOQUINOL**

The residue (216 g, 0.326 mol) from the EtOAc washing of the crude reaction material (64% -ol, 20% -one, 16% MePPh₃Br) as in the first part of Example 3 was dried *in vacuo* then dissolved in methanol (700 mL). A solution of 30% aqueous H₂O₂ (70 mL, 0.618 mol) and pyridine (134 mL) were added and the mixture was stirred 21 hrs at room temperature. The methanol was then evaporated *in vacuo* and the crude mixture was dissolved in dichloromethane (1.6 L) and extracted with 2% aqueous HBr (4 x 700 mL). The organic layer was dried over MgSO₄ and added directly to a silica gel bed (1.2 kg (Merck type 9385) dry packed, 65mm deep by 245/230 mm wide in a sintered glass funnel). The silica gel was washed using a slight vacuum with dichloromethane (1.0 L), then 5% rectified spirits in dichloromethane (10.0 L) and 10% rectified spirits in dichloromethane (3.0 L).

Evaporation of the 5% rectified spirits in dichloromethane solution gave of pure mitoQuinone (166.2 g, 76.9%). ¹H NMR (299.9 MHz) 7.7-7.9 (m, 15H, -P⁺Ph₃), 3.98 (s, 6H, 2x -OCH₃), 3.85-3.95 (m, 2H, -CH₂-P⁺Ph₃), 2.40 (t, J=7.8Hz, 2H, ubiquinone-CH₂-), 2.00 (s, 3H, CH₃). ³¹P NMR (121.4 MHz) 25.7 ppm.

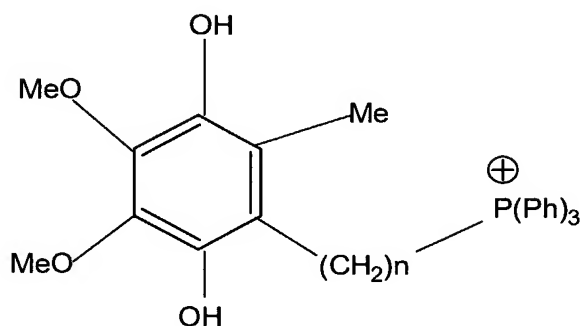
Evaporation of the 10% rectified spirits in dichloromethane solution gave a 29:71 mixture of mitoQuinone and methyltriphenylphosphonium bromide (19.2 g).

MitoQuinone (0.31 g, 0.47 mmol) was dissolved in methanol (10 ml) and stirred under argon at room temperature. Sodium borohydride (0.1 g) was added to the stirred solution which went light yellow and the mixture was stirred for 30 minutes. A solution of 48 % HBr was then added dropwise until gas evolution finished and the methanol was then evaporated *in vacuo*. The residue was dissolved in a mixture of dichloromethane (5 ml) and H₂O (5 ml) and the organic layer was collected. The aqueous phase was extracted with a further portion of dichloromethane (5 ml). The combined organic fractions were dried over Na₂SO₄ and the solvents evaporated *in vacuo* to give a yellow foam (0.305 g, 97 %). ¹H NMR showed no evidence for a peak at 2.045 ppm indicating <3% residual mitoQuinone impurity.

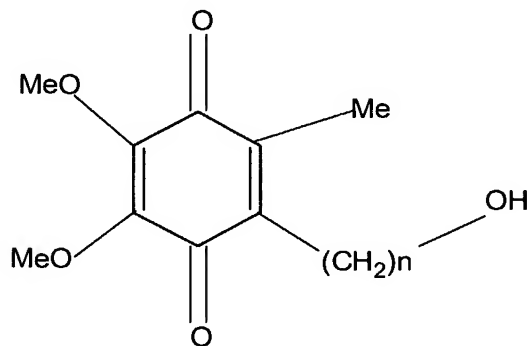
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CLAIMS:

1. A method of synthesis of a compound with a moiety or the moiety of the formula

(Formula II)

(and/or its quinone form) where n is an integer from 6 to 40 which comprises or includes the preparation or obtaining of a compound of formula

(Formula I)

(and/or its quinol form) and

its subsequent reaction in the presence of Ph_3PHX and Ph_3P , where X is a halogen atom selected from Br, I and Cl.

2. A method of claims 1 wherein X is Br.

3. A method of claim 1 or 2 wherein the product compound includes Br.

4. A method of claim 1 or 2 wherein n is up to 25.

5. A method of claim 1 wherein n is 10.

6. A method of claim 1 wherein the ratio of the compound of Formula 1 to Ph_3P and the ratio of the Ph_3PHX to Ph_3P are each substantially stoichiometric.

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7. A method of claim 6 wherein X is Br.
8. A method of claim 1 wherein the reaction(s) is (are) maintained below 80°C.
9. A method of claim 1 wherein the compound of Formula I is largely or exclusively in its quinone form.
- 5 10. A method of claim 1 wherein the compound with a moiety of Formula II is largely or exclusively in its quinol form.
11. A method of claim 1 followed by a purification process.
12. A method of claim 10 followed by a reduction of any quinone form material to its quinol form.
- 10 13. A compound with a moiety of Formula II when produced by a process of claim 1.
14. A compound of claim 13 when produced by a process of any one of claims 2 to 12.
15. A compound of claim 13 or 14 which is mitoquinol and/or mitoquinone.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/NZ02/00154

A. CLASSIFICATION OF SUBJECT MATTER												
Int. Cl. ⁷ : C07F 09/54												
According to International Patent Classification (IPC) or to both national classification and IPC												
B. FIELDS SEARCHED												
Minimum documentation searched (classification system followed by classification symbols)												
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Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) STN Substructure Search												
C. DOCUMENTS CONSIDERED TO BE RELEVANT												
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.										
A	Journal of Biological Chemistry, Vol. 276, No. 7, 16 February 2001, Kelso et al, "Selective Targeting of a Redox-active Ubiquinone to Mitochondria within Cells", pp4588-4596 (&US 6,331,532 A)											
A	Free Radical Biology & Medicine, Vol. 28, No. 10, 2000, Coulter et al, "Mitochondrially Targeted Antioxidants and Thiol Reagents", pp1547-1554											
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